

CLAIM AMENDMENTS

1-30. (Canceled)

31. (Currently amended): A method for stereoselectively producing an alpha-substituted carboxylic acid, the method comprising

- (a) providing an aldehyde or a ketone;
- (b) providing a cyanide-containing compound;
- (c) providing an ammonia-containing compound or a compound comprising an ammonium salt or an amine;
- (d) providing a composition comprising a nitrilase, wherein the nitrilase has an amino acid sequence consisting of SEQ ID NO:2[[],] or SEQ ID NO:4, or an enzymatically active fragment thereof, wherein the fragment retains the enzymatic function of SEQ ID NO:2[[],] or SEQ ID NO:4;
- (e) contacting the aldehyde or ketone of step (a) with a cyanide-containing compound of step (b) and an ammonia-containing compound or a compound comprising an ammonium salt or an amine of step (c) such that an amino nitrile or a cyanohydrin intermediate is produced; and
- (f) contacting the amino nitrile or cyanohydrin intermediate of step (e) with the composition of step (d) such that the nitrilase stereoselectively hydrolyzes the amino nitrile or cyanohydrin intermediate to produce an alpha-substituted carboxylic acid.

32. (Currently amended): A method for stereoselectively producing an alpha-substituted carboxylic acid, the method comprising

- (a) providing a composition comprising an amino nitrile or a cyanohydrin;
- (b) providing a composition comprising a nitrilase, wherein the nitrilase has an amino acid sequence consisting of SEQ ID NO:2[[],] or SEQ ID NO:4, or a enzymatically active fragment thereof, wherein the fragment retains the enzymatic function of SEQ ID NO:2[[],] or SEQ ID NO:4; and

(c) contacting the amino nitrile or cyanohydrin of step (a) with the composition of step (b) such that the nitrilase stereoselectively hydrolyzes the amino nitrile or cyanohydrin intermediate to produce an alpha-substituted carboxylic acid.

33-35. (canceled)

36. (Currently amended): A method for stereoselectively producing an alpha-amino acid, the method comprising

- (a) providing an aldehyde or a ketone;
- (b) providing a cyanide-containing compound and ammonia;
- (c) providing a nitrilase, wherein the nitrilase has an amino acid sequence consisting of SEQ ID NO:2[[],] or SEQ ID NO:4, or a enzymatically active fragment thereof, wherein the fragment retains the enzymatic function of SEQ ID NO:2, or SEQ ID NO:4;
- (d) contacting the aldehyde or ketone of step (a) with the cyanide-containing compound and ammonia of step (b) such that an amino nitrile is produced; and
- (e) contacting the amino nitrile of step (d) with the nitrilase of step (c) such that the nitrilase stereoselectively hydrolyzes the amino nitrile to produce an alpha-substituted amino acid.

37. (Currently amended): The method of claim 31, ~~claim~~ 32 or ~~claim~~ 36, wherein the reaction takes place in a single reaction vessel.

38-43. (canceled)

44. (Currently amended): A method for stereoselectively producing an alpha-substituted carboxylic acid, said method comprising

contacting an aldehyde or ketone with a cyanide-~~comprising~~ containing compound and an ammonia-~~comprising~~ containing compound, an ammonium salt or an amine, and

hydrolyzing stereoselectively the resulting amino nitrile or cyanohydrin intermediate with a nitrilase, wherein the nitrilase hydrolyzes the reaction components to stereoselectively produce an alpha-substituted carboxylic acid and wherein the nitrilase has (i) an amino acid sequence having at

least 70% sequence identity to an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4 wherein the amino acid sequence retains the same biological activity as SEQ ID NO:2 or SEQ ID NO:4, or (ii) is encoded by a nucleic acid having at least 70% sequence identity to an nucleic acid sequence consisting of SEQ ID NO:1 or SEQ ID NO:3, wherein the nucleic acid encodes an enzyme that retains the same enzymatic activity as the enzyme encoded by the nucleic acid sequence from which it varies.

45-48. (canceled)

49. (Currently amended): A method for stereoselectively producing an alpha-substituted carboxylic acid, the method comprising

providing a composition comprising a nitrilase, wherein the nitrilase has an amino acid sequence consisting of SEQ ID NO:2[[],] or SEQ ID NO:4, or a enzymatically active fragment thereof, wherein the fragment retains the enzymatic function of SEQ ID NO:2[[],] or SEQ ID NO:4; and

contacting reaction components with the composition such that the nitrilase stereoselectively hydrolyzes the reaction components to produce an alpha-substituted carboxylic acid,

wherein the reaction components are an aldehyde or ketone, a cyanide-containing compound, and an ammonia-containing compound, ammonia salt, or amine.

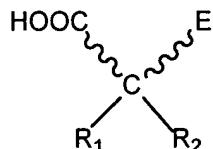
50. (Currently amended): A method for stereoselectively producing an alpha-substituted carboxylic acid, said method comprising hydrolyzing stereoselectively the reaction components with a nitrilase,

wherein the reaction components are an aldehyde or ketone, a cyanide-containing compound, and an ammonia-containing compound, ammonia salt, or amine,

wherein the nitrilase has (i) an amino acid sequence having at least 70% sequence identity to an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4 wherein the amino acid sequence retains the same biological activity as SEQ ID NO:2 or SEQ ID NO:4, or (ii) is encoded by a nucleic acid having at least 70% sequence identity to an nucleic acid sequence consisting of SEQ ID NO:1 or SEQ ID NO:3,

wherein the nucleic acid encodes an enzyme that retains the same enzymatic activity as the enzyme encoded by the nucleic acid sequence from which it varies.

51. (New) The method of claims 31, 32, or 36, wherein said α -substituted carboxylic acid has the following structure:



wherein:

R₁ and R₂ are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally R₁ and R₂ are linked to cooperate to form a functional cyclic moiety, and

E is -N(R_x)₂ or -OH, wherein each R_x is -H or lower alkyl.

52. (New) The method of claim 31, 32, or 36, wherein said α -substituted carboxylic acid is an α -amino acid.

53. (New) The method of claim 51, wherein at least one of R₁ and R₂ is substituted or unsubstituted aryl.

54. (New) The method of claim 52, wherein said α -amino acid is D-phenylalanine, D-phenylglycine, or L-methylphenylglycine.

55. (New) The method of claim 52, wherein said α -amino acid bears a substituted or unsubstituted alkyl side chain.

56. (New) The method of claim 52, wherein said α -amino acid is L-tert-leucine, D-alanine, or D-hydroxynorleucine.

57. (New) The method of claim 31, 32, or 36, wherein said α -substituted carboxylic acid is an α -hydroxy acid.

58. (New) The method of claim 57, wherein said α -hydroxy acid is (S)-cyclohexylmandelic acid, mandelic acid or 2-chloro mandelic acid.

59. (New) The method of claim 31 or 36, wherein said cyanide-containing compound comprises a metal cyanide or a gaseous cyanide.

60. (New) The method of claim 59, wherein said cyanide-containing compound comprises an alkali cyanide.

61. (New) The method of claim 59, wherein said cyanide-containing compound is sodium cyanide.

62. (New) The method of claim 51, wherein said ammonia salt has the formula $\text{NH}_2(\text{R})_2^+ \text{X}^-$, wherein each R is independently -H or lower alkyl, and X is a counter ion.

63. (New) The method of claim 62, wherein X is a halide.

64. (New) The method of claim 63, wherein said halide is Cl^- .

65. (New) The method of claim 64, wherein said ammonia salt is $\text{NH}_4^+ \text{Cl}^-$.